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13. ABSTRACT *(Maximum 200 words)*

The goal of this project was to test a concept for addressing multielement fluorescence-based biosensor arrays through a single optical fiber. Multielement chemical sensor arrays are potentially advantageous for several applications where fiber optic sensors may be used, especially for simultaneous multianalyte determinations *in situ*. Unfortunately, use of multiple fibers or fiber bundles, or employing an optical switch between array elements at the distal end near the transducers are cumbersome and infeasible for many applications; thus a means was sought to excite and collect fluorescence from individual array elements using an active device at the proximal end of the fiber.

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FINAL REPORT

Grant No.: N00014-98-1-0475

PRINCIPAL INVESTIGATOR: Richard B. Thompson

INSTITUTION: University of Maryland

GRANT TITLE: Biosensor Array Remotely Addressable Through a Single Optical Fiber

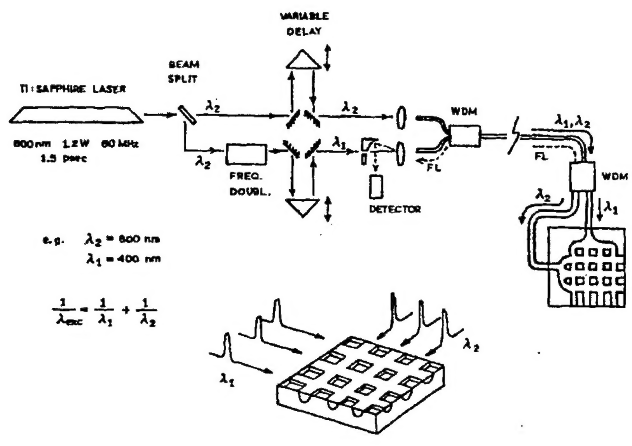
AWARD PERIOD: March 2, 1998 to March 1, 1999

OBJECTIVE: To test a concept for exciting and collecting fluorescence from a two dimensional array of sensor transducers at the distal end of a single optical fiber; a key objective is that there should be no active devices in the transducer array.

APPROACH: Fiber optic chemical sensors have important advantages for many applications, especially sensing in malign, flammable, remote, or electrically noisy environments. Such environments include the deep ocean, machinery spaces, and fuel tanks. A key attribute of these sensors is that the sensor transducer which is exposed to the analyte is a passive device connected to the rest of the instrument by an optical fiber. For some applications sensor arrays comprising groups of transducers also have important advantages, especially multicomponent analysis. While in principle multiple sensor transducers could be monitored each with its own optical fiber, this approach becomes cumbersome with arrays having more than a few transducers. Similarly, an optical switch could be used at the distal end of a single optical fiber to serially interrogate each transducer, but this approach is also cumbersome, and requires an (electrically powered) switch be located at the distal end, which negates some of the advantages of the sensor. We sought a means to connect the array to the optoelectronic device at the proximal end of a single optical fiber, while at the same time having no active switching device at the distal end with the array.

The approach we proposed to address this issue is illustrated in Figure 1. The approach takes advantage of a phenomenon called two color/two photon excitation of fluorescence. This phenomenon is akin to classic two photon-excited fluorescence, wherein two photons (in a sense) combine to form a virtual excitation photon having

half the wavelength, which can excite fluorescence; thus sufficiently intense (high peak power) infrared excitation may excite fluorescence in a fluorophore which only absorbs at visible wavelengths. High peak powers are best obtained by focusing picosecond light pulses from a modelocked titanium sapphire laser into a small volume with a high numerical aperture lens. Two color /two photon excitation occurs when two photons of different colors combine to excite fluorescence.



PULSES "COLLIDE" AT A UNIQUE POINT
GIVE 2-COLOR, 2-PHOTON EXCITATION

The heart of the concept was to put transducers (spots of fluorescent indicator) on the surface of a two-dimensional lithium niobate waveguide, and arrange to have trains of pulses of light "collide" at particular intersections in the waveguide; where the pulses were coincident, two color / two photon excitation could occur and be capable of exciting the fluorescence of the spots. Particular transducers would be excited by timing the arrival of pulses in each dimension to collide by adjusting a delay line. Light of one color would be demultiplexed from the single fiber and launched in the waveguides parallel to one axis using a simple 1 x X splitter, and similarly for the waveguides parallel to the other axis.

Issues in bringing this concept to fruition were the construction of the waveguide itself, and its ability to keep the pulses from spreading too much spatially or temporally; the inefficiency of evanescent wave excitation of fluorophores on the surface of the waveguide; and the difficulty of choosing wavelengths where the test fluorophores would be excited by two color / two photon excitation, but not two photon (one color) excitation from the shorter excitation wavelength of the two colors.

Finally, the current commercial unavailability of multiplexer/demultiplexers at the relevant wavelengths is an issue.

The waveguide was custom made by Edgar Mendoza of Physical Optics Corp. (now Intelligent Optical Systems) of Torrance CA. It is approximately 2 x 2 cm, made of lithium niobate, with waveguides formed by indiffusion of titanium ions. Several different fluorophores were tried, including p-terphenyl, Dapoxyl sulfonamide, and Bis-MSB, based on Ignacy Gryczynski's original report. Excitation was provided by a Ti:sapphire laser operated at 823 nm, together with its frequency doubled output at 412 nm; average power was about 800 mW before frequency doubling, and pulse duration was approximately 1.5 psec.

ACCOMPLISHMENTS: We did not succeed in our goal of observing fluorescence at the distal end array excited by two color / two photon excitation. The experimental setup we used was very similar to that diagramed in Figure 1, except that light of the two different colors was launched directly into the two sides of the array, not launched into a fiber and demultiplexed at the distal end.

CONCLUSIONS: It seems likely that a two dimensional array device of the type we tried would need more sophisticated design than that available within our resources, and perhaps greater laser power as well. We note that for our fluorescence lifetime-based sensor transducers in many cases any of a variety of fluorescent labels are workable, and thus the label could be chosen for its suitability for two color / two photon excitation. A better approach might be to try a one dimensional array on the surface of a waveguide (optical fiber) whose properties can be better optimized, to guide design of the two dimensional waveguide.

SIGNIFICANCE: The ability to remotely address an array of (optical) sensor transducers through a single optical fiber would be very significant for some applications. For instance, for monitoring multiple analytes deep in the water column (at kilometer depths), a transducer array that is entirely passive (e.g., no moving parts, no electricity required) would be very desirable. The dielectric waveguide is relatively insensitive to pressure, and consequently no heavy, leak-prone pressure housing for the sensor array would be necessary, nor are leak-prone housing penetrations necessary.

PATENT INFORMATION: Invention disclosure 1452RT filed with University of Maryland Office of Research and Development;

action is pending.

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